A Comparative Analysis of the Sulfur-rich Proteins of Durum and Bread Wheats—Their Possible Functional Properties

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ABSTRACT

Comparison of the solubility and the electrophoretic characteristics of sulfur-rich (chloroform/methanol-soluble) proteins of durum and bread wheats showed considerable differences. Most of these proteins were readily extracted from bread wheat with the albumin-globulin fraction, whereas only some of them were extracted from durum wheat under similar conditions. The remaining sulfur-rich protein fraction of durum wheat could be separated from the other glutenin components with Na-tetradecanoate. In bread wheat, some of the sulfur-rich proteins became bound to other gluten components during dough formation, probably through hydrophobic interactions. They were extracted from gluten partly with the gliadin and partly with the glutenin fraction. Results suggested that these proteins, as part of the gluten network, may have a functional role in the formation of dough.

A highly significant correlation was found between the cooking quality of durum wheat pasta and the sulfhydryl (-SH) plus disulfide (S-S) contents of DSG proteins (durum wheat, sulfur-rich glutenins) (Kobrehel and Alary 1989a). These proteins are composed of two fractions, DSG-1 and DSG-2, with molecular masses of 14.1 and 17.1 kilodaltons, respectively, determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Kobrehel et al 1988, Kobrehel and Alary 1989b). They were extracted from semolinas of durum wheats (*Triticum durum* Desf.), using low concentrations of Na-tetradecanoate, subsequent to the extraction of albumins, globulins, and gliadins. The N-terminal amino acid sequences of DSG-1 and DSG-2 (Kobrehel and Alary 1989b) were found to be similar to the N-terminal amino acid sequences of the CM proteins (chloroform/methanol-soluble) CM16 and CM3, respectively (Barber et al 1986, Shewry et al 1984), suggesting identity between these proteins. The characterization of a cDNA encoding the DSG-1 gave further evidence that the DSG and the CM proteins belong to the same family (Gautier et al 1989, 1990).

The CM proteins of bread wheat are readily extracted with salt solutions (Paz-Ares et al 1983, Barber et al 1986), whereas the DSG proteins are much less soluble under these conditions (Kobrehel et al 1988). It seems that CM3 and CM16 are components of tetrameric α-amylase inhibitors (Garcia-Olmedo et al 1987). No functional properties have been detected for the CM proteins by technological methods currently in use.

On the other hand, the importance of -SH groups and/or S-S bonds in the functional properties of bread wheat (*Triticum aestivum* L.) proteins has been reported by many authors (Sullivan 1963, Stevens 1966, Lee and Lai 1967, Tsen and Bushuk 1969, Huebner et al 1977, Archer 1979, Ewart 1988). However, these studies were not performed on isolated protein fractions, and, in general, investigations concerning the molecular basis of baking quality have focused mostly on gliadins and glutenins.

On the basis of previous research on the functional properties of DSG proteins in durum wheat pasta products, it can be hypothesized that sulfur-rich CM (DSG-type) proteins may play a functional role in the quality of bread wheat. We compared the conditions for extraction and some other characteristics of DSG proteins in durum and bread wheats and made some preliminary investigations on the possible functional role of proteins in bread wheat. The first results of these studies are presented in this paper.

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MATERIALS AND METHODS

Wheat Samples

Durum wheat semolina of cultivars Mondur and Kidur and bread wheat flour of cultivars Talent and Hardi were used.

Preparation of Gluten

Wet gluten was prepared from durum wheat semolina or from bread wheat flour by the hand washing procedure (Mauze et al 1972), except that distilled water was used for washing instead of tap or salted water.

Extraction of Proteins

From both semolina and flour, albumins plus globulins and gliadins were extracted sequentially with 0.5*M* NaCl and 68% (v/v) ethanol under the conditions described by Alary and Kobrehel (1987). For 1 g of semolina or flour, 10 ml of solvent was used. Two glutenin fractions (Glu-1 and Glu-2) were extracted sequentially from the residues, Glu-1 with 3.75 mg and Glu-2 with 80 mg of Na-tetradecanoate for the initial amount of 1 g of semolina or flour. All extractions were for 15 hr at 4°C, and the supernatents were recovered by centrifugation at 38,000 × *g* for 30 min at 4°C. From gluten, the sequential extraction of proteins started with the extraction of gliadins. All the experimental conditions were as for semolina and flour.

Column Chromatography of Proteins

Protein extracts were separated by molecular sieving chromatography on Biogel P-30, mesh size 100-200 (Bio-Rad Laboratories). The column (2.5 × 100 cm, packed height of 98 cm) was equilibrated with 0.05*M* acetic acid. About 50 mg of freeze-dried protein was dissolved in 0.05*M* acetic acid, filtered through a folded filter (Macherey-Nagel 7131) and applied to the column. Filtration was performed only in the case of the glutenin fractions. The flow rate was 25 ml/hr, the eluate was monitored at 280 nm, and fractions of 5 ml were collected. The chromatographic separations were carried out at room temperature.

Electrophoresis of Proteins

Proteins were analyzed either after reduction or without reduction using SDS-PAGE as described by Payne and Corfield (1979). Two-dimensional electrophoresis, isoelectric focusing (IEF) × SDS-PAGE, was performed as described by O'Farrell (1975). For the IEF separations, a gradient from pH 4 to 9 was used.

Determination of -SH Plus S-S Content

The sulfhydryl plus disulfide contents of the protein fractions were determined amperometrically under the conditions described by Alary and Kobrehel (1987).

Determination of Protein Content

A semiautomatic Kjeldahl method was used as described by Feillet (1967). Protein content was calculated as N × 5.7.

RESULTS AND DISCUSSION

Sulfhydryl Plus Disulfide Content of the Protein Fractions

The –SH plus S–S content of the protein fractions of bread wheat, with the exception of Glu-1 (glutenin fraction extracted using a small amount of Na-tetradecanoate), was considerably higher than in the corresponding protein fractions of durum wheat (Table I). For the sequential extraction conditions used, Glu-1 of durum wheat contained sulfur-rich proteins. In bread wheat, however, the –SH plus S–S content was higher in glutenin fractions extracted with larger amounts of Na-tetradecanoate (Glu-2) than in Glu-1.

Electrophoretic Composition of Sequentially Extracted Proteins

As shown in Figure 1, in agreement with earlier results (Kobrehel et al 1988), Glu-1 extracted from durum wheat semolina was composed mostly of DSG (=CM) proteins. The composition of Glu-1 obtained from bread wheat flour was considerably different. Unlike durum wheat, sulfur-rich (CM-type) proteins were not extracted from bread wheat with the Glu-1 fraction, showing that these proteins of bread wheat and of durum wheat have different solubility properties. The absence of CM proteins in the bread wheat Glu-1 fraction suggests different types of interactions with other protein and/or nonprotein components of the flour than those that occur in durum wheat semolina.

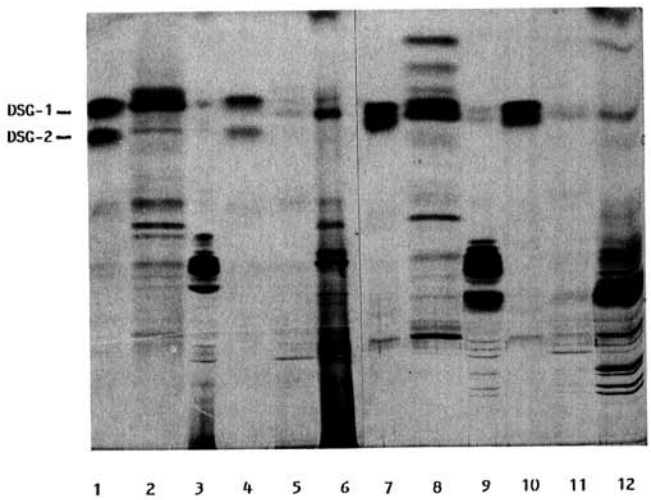

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sequentially extracted protein fractions from durum wheat cultivar Mondur (slots 1, 4, 7, and 10) and from bread wheat cultivar Talent (all the other slots). For slots 1–6, proteins were not reduced, and for slots 7–12, proteins were reduced before electrophoresis. Extractions were performed with 0.5*M* NaCl (2 and 8), 68% ethanol (3 and 9), 3.75 mg of Na-tetradecanoate (1, 4, 5, 7, 10, and 11), and 80 mg of Na-tetradecanoate (6 and 12). Origin at bottom of figure.

When the proteins of Glu-1 were not reduced, only traces of bands with mobilities similar to those of DSG proteins were observed. The major protein band in that area had an intermediate mobility compared with the mobilities of DSG-1 and DSG-2. In bread wheat, the electrophoretic pattern of low molecular weight proteins in Glu-2 was similar to Glu-1, although the relative intensity of one major band was higher in Glu-2 than in Glu-1. In durum wheat semolina, the –SH plus S–S content was much higher in proteins with lower molecular weight than in proteins with higher molecular weight (Kobrehel et al 1988). In the Glu-1 fraction of bread wheat, the proportion of low molecular weight proteins was much lower than in durum wheat, as judged by the intensity of electrophoretic bands, which would explain the lower –SH plus S–S content of this fraction.

Electrophoresis of reduced proteins showed that in the glutenin fractions extracted from bread wheat, there was no band with the mobility of DSG-2. In both extracts (Glu-1 and Glu-2), the major low molecular weight protein had an intermediate mobility compared with the mobilities of DSG proteins, and only a trace of a band was present with mobility similar to DSG-1. In contrast to Glu-1 and Glu-2, the albumin-plus-globulins fraction extracted from bread wheat contained proteins having the mobility of DSG proteins. This is in agreement with the results reported in the literature that CM proteins from bread wheat and from other cereals can be extracted with salt solutions (Paz-Ares et al 1983, Barber et al 1986).

Chromatographic Separation of Proteins and Analysis by SDS-PAGE

For further comparison, proteins were fractionated by molecular sieving chromatography under the conditions used to isolate DSG proteins (Kobrehel and Alary 1989b). These analyses were performed on albumins plus globulins and on Glu-1 of bread wheat, and the results were compared to those obtained on Glu-1 of durum wheat. The elution profiles of these fractions are shown in Figure 2. The Glu-1 of durum wheat yielded three major peaks. SDS-PAGE analysis showed that peak 1 was composed of glutenins with higher apparent molecular masses than those of DSG proteins (results not shown), whereas peaks 2 and 3 contained DSG-2 and DSG-1, respectively (Fig. 3). For the Glu-1 of bread wheat the elution profile showed only very small peaks having retention times close to those of DSG proteins (Fig. 2). These peaks yielded only very faint bands on SDS-PAGE (Fig. 3).

The albumin-globulin fraction of bread wheat contained considerable amounts of low molecular weight proteins with retention times on the P-30 column close to that of DSG proteins (Fig. 2). SDS-PAGE analysis without reduction showed that these peaks were composed of four major proteins. Two of these bands had electrophoretic mobilities similar to those of DSG proteins (Fig. 4, shown by arrows).

SDS-PAGE of reduced albumin-globulin and Glu-1 fractions of bread wheat, after further resolution by column chromatography, showed less similarity to DSG proteins than when not reduced. As shown in Figure 3, the small peaks of Glu-1 contained two bands, one having mobility like DSG-1 and another with an intermediate mobility between DSG-1 and DSG-2. Neither Glu-1 nor the albumin-globulin fractions of bread wheat contained

TABLE I
The Total Sulfhydryl plus Disulfide Content in the Protein Fractions Extracted Sequentially from Two Bread Wheats and Two Durum Wheats

Protein Fractions	Protein Extracted (% of total protein)				Total–SH plus S–S (μmol/g of protein)			
	Bread Wheats		Durum Wheats		Bread Wheats		Durum Wheats	
	Hardi	Talent	Mondur	Kidur	Hardi	Talent	Mondur	Kidur
Albumins plus globulins	16	18	17	21	230	272	137	135
Gliadins	24	32	38	41	135	129	103	102
Glu-1[a]	1	4	2	3	92	61	184	132
Glu-2[b]	19	28	27	29	124	94	92	88

[a] Extracted with 3.75 mg of Na-tetradecanoate per gram of flour or semolina.
[b] Extracted with 80 mg of Na-tetradecanoate per gram of flour or semolina.

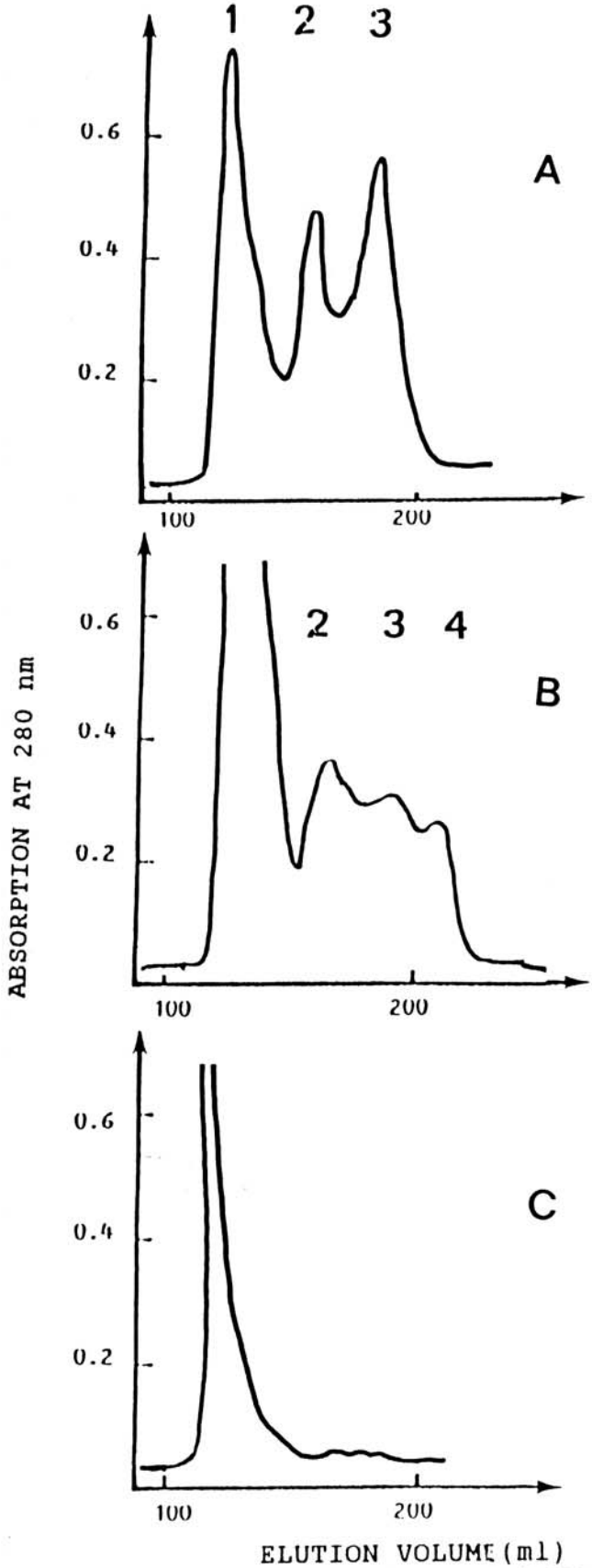

Fig. 2. Gel filtration elution profiles of proteins extracted with 3.75 mg of Na-tetradecanoate subsequent to the extraction of albumins, globulins, and gliadins from durum wheat semolina, cultivar Mondur (**A**), and from bread wheat flour, cultivar Talent (**C**), compared with the albumin-globulin fraction extracted with 0.5*M* NaCl from bread-wheat flour (**B**).

a band having exactly the same mobility as DSG-2. This may be of little relevance since the electrophoretic mobility of DSG-2 among durum wheats showed intervarietal differences (Kobrehel and Alary 1989a). In peak 3 of the albumin globulin fraction from bread wheat, the most intense band, with or without reduction of the proteins, had the same mobility as DSG-1. However, as shown in Figure 2, the retention time of peak 3 was longer than that for peak 3 of durum wheat, which contained DSG-1. Peak 2 of the albumin-globulin fraction showed, without reduction of the proteins, two bands with mobilities like those of DSG-1 and DSG-2, whereas after reduction only one major band was seen; it had a mobility intermediate between DSG-1 and DSG-2. This suggests that peak 2 and peak 3 proteins with similar mobilities before reduction (Fig. 4) are not identical.

Two-Dimensional Electrophoretic Analyses (IEF × SDS-PAGE)

DSG proteins showed heterogeneity on IEF; both DSG-1 and DSG-2 were composed of three main spots with one of the spots much more intense than the other two (Fig. 5). Proteins of bread

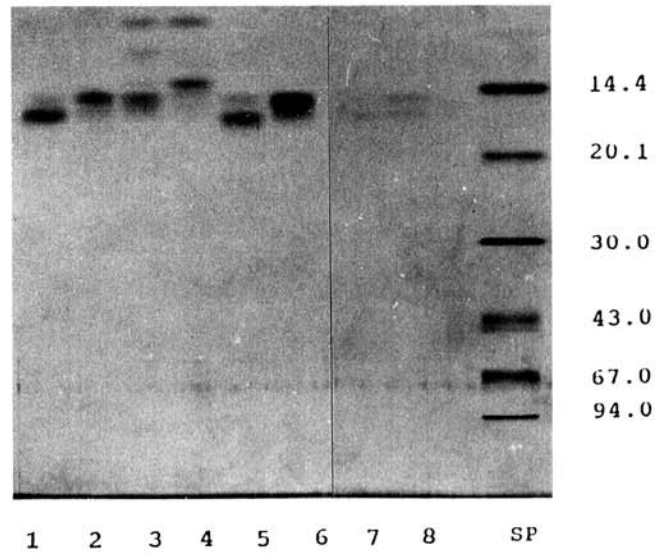

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins fractionated previously on Biogel P-30 (Fig. 2). Slot numbers are given in parentheses. Proteins extracted with 0.5*M* NaCl from bread wheat: peak 2 (**1**), peak 3 (**2** and **3**), peak 4 (**4**). Proteins extracted with 3.75 mg of Na-tetradecanoate from durum wheat: peak 2 (**5**), peak 3 (**6**), and from bread wheat for the same elution volume as peaks 2 and 3 of durum wheat (**7** and **8**). SP = Standard proteins (apparent $M_r \times 10^{-3}$ shown). Samples were reduced before electrophoresis.

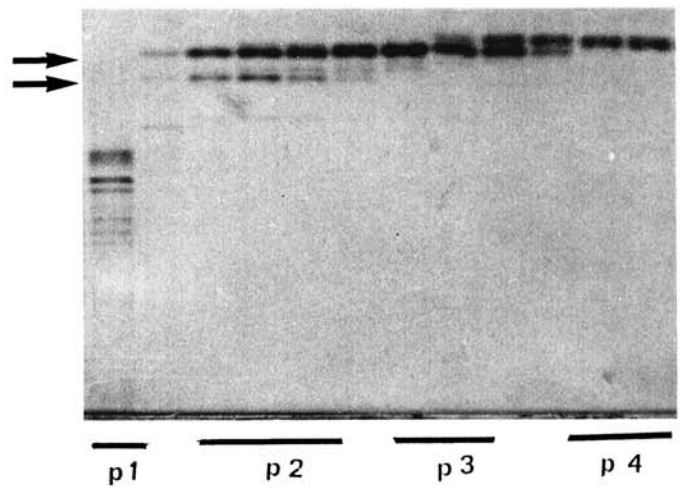

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins extracted with 0.5*M* NaCl from bread wheat, cultivar Talent, and fractionated on Biogel P-30 (B in Fig. 2.). Proteins were not reduced.

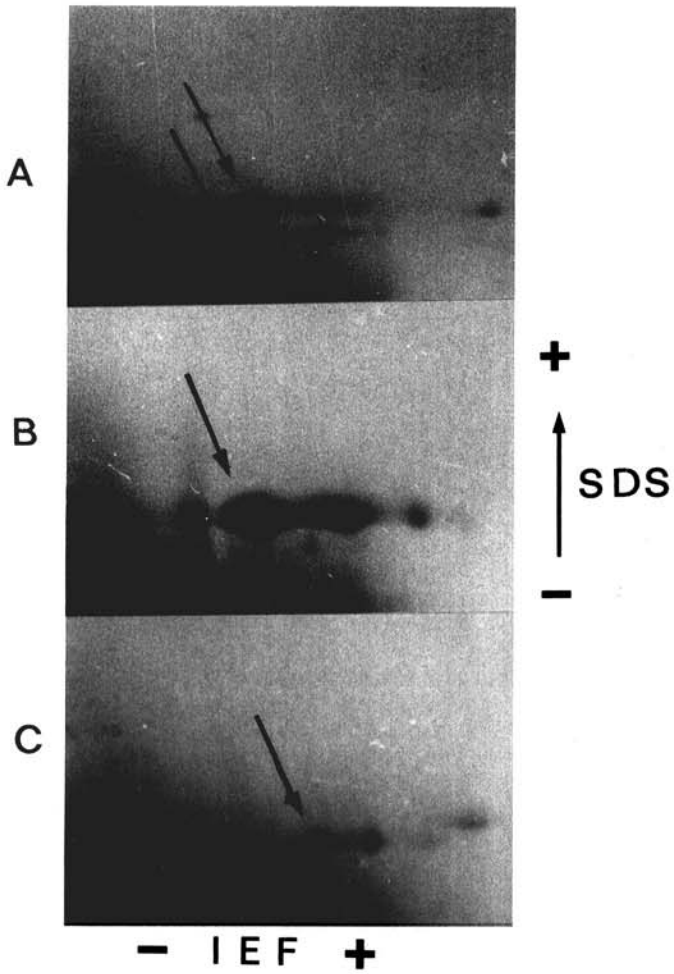

Fig. 5. Two-dimensional electrophoresis (first dimension, isoelectric focusing (IEF); second dimension, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis) of proteins fractionated previously on Biogel P-30. **A,** Peak 2 of the albumin-globulin fraction (2 in Fig. 2B and peak 2 in Fig. 4); **B,** DSG-2 (peak 2 in Fig. 2A and slot 5 in Fig. 3); and **C,** DSG-1 (peak 3 in Fig. 2A and slot 6 in Fig. 3).

wheat isolated as peak 2 in Figure 2 and, which on SDS-PAGE had mobilities similar to those of DSG proteins, were also more heterogeneous on IEF than on SDS-PAGE (Fig. 5). When they were focused together with DSG-1 or with DSG-2 (results not shown here), in each case only one of the spots of the DSG proteins was superimposed with one of the spots of the proteins contained in peak 2. Spots found in both the DSG proteins and the albumin-globulin fraction of bread wheat are shown by the arrows. The other spots of the albumins-globulins (or CM proteins) of bread wheat and of the DSG proteins had different electrophoretic characteristics.

According to all the preceding results, DSG-1 and DSG-2 from durum wheat and CM-type proteins from bread wheat showed considerable differences in their respective solubilities and their chromatographic and electrophoretic characteristics. On the other hand, analyses did not show noticeable differences between the two durum wheat cultivars.

Amino Acid Analyses

Table II shows the amino acid compositions of durum wheat protein fractions of Glu-1 separated on Biogel P-30 (Fig. 2) and of bread wheat proteins isolated from the albumin-globulin fraction, whose electrophoretic characteristics were similar to those of DSG proteins (peaks 2, 3, and 4 in Fig. 2) and peak 1 of the Glu-1 fraction (Fig. 2). Peaks 2, 3, and 4 of the albumin-globulin extract are composed of proteins rich in cysteine. The amino acid compositions of the three fractions are similar but when compared to those of DSG proteins, some differences are noticeable. For instance, they have higher aspartic acid but lower glycine and methionine contents than the DSG proteins. Yet they seem to be CM-type proteins; their amino acid compositions and those reported for CM proteins are closely related (Salcedo et al 1978, Barber 1986). Their amino acid compositions are also comparable to those of S-proteins (Zawistowska et al 1986). On the other hand, the amino acid compositions of peak 1 of Glu-1 from bread and durum wheats were different from those of CM-type proteins. These differences were considerable for most of the amino acids. They contained much less cysteine and more glutamic acid (or glutamine) and proline.

Analyses on Gluten

The glutenin fraction Glu-1 extracted from bread wheat gluten, in comparison with Glu-1 extracted from the flour that was used to prepare the gluten samples, contained a higher proportion of CM-type proteins (Fig. 6). The electrophoretic mobilities of the two major low molecular weight proteins were similar to those

TABLE II
Amino Acid Compositions of Proteins Extracted from Bread and Durum Wheats and Fractionated on Biogel P-30[a]
(mol % of total protein)

	Bread Wheat Cultivar Talent					Durum Wheat Cultivar Mondur		
Amino Acid	Glu-1 P-1	Glu-1 P-2-3	AG P-2	AG P-3	AG P-4	Glu-1 P-1	DSG-1	DSG-2
Alanine	1.5	6.2	4.4	4.7	4.7	2.9	5.1	6.5
Arginine	2.1	7.8	7.5	7.4	7.8	2.8	5.9	5.5
Aspartic acid[b]	1.8	6.6	6.3	8.4	8.6	3.1	3.0	1.8
Cysteine	1.5	6.8	8.1	10.6	7.0	3.1	6.0	5.5
Glutamic acid[b]	46.1	16.1	18.1	16.2	14.5	39.0	17.3	14.2
Glycine	2.5	4.2	3.9	4.2	4.4	2.0	7.2	7.5
Histidine	1.9	1.8	1.7	1.8	1.5	2.6	0.8	1.2
Isoleucine	2.9	2.7	3.0	3.2	2.6	3.4	3.9	4.0
Leucine	5.3	7.8	8.3	6.8	7.2	6.4	8.1	10.1
Lysine	0.6	2.9	1.8	2.8	2.4	1.1	1.6	1.4
Methionine	0.1	0.6	0.4	1.5	0.9	0.2	3.9	3.3
Phenylalanine	6.2	3.1	3.4	2.3	2.3	5.6	2.9	4.2
Proline	16.0	14.3	13.8	11.9	16.2	15.5	12.2	12.6
Serine	4.7	4.7	4.9	4.8	4.7	4.6	5.8	6.3
Threonine	2.0	3.5	4.1	4.1	4.1	2.3	6.3	4.4
Tyrosine	2.0	4.7	4.3	4.5	4.1	2.2	4.1	4.0
Valine	2.5	5.7	5.5	5.5	5.7	3.6	5.7	7.5

[a] Glu-1 P-1 = peak 1 of the Glu-1 fraction, Glu-1 P-2-3 = peaks 2 and 3 of the Glu-1 fraction, AG = peaks 2, 3, and 4 of the albumin-globulin fraction. Tryptophan was not determined.
[b] Include asparagine and glutamine, respectively.

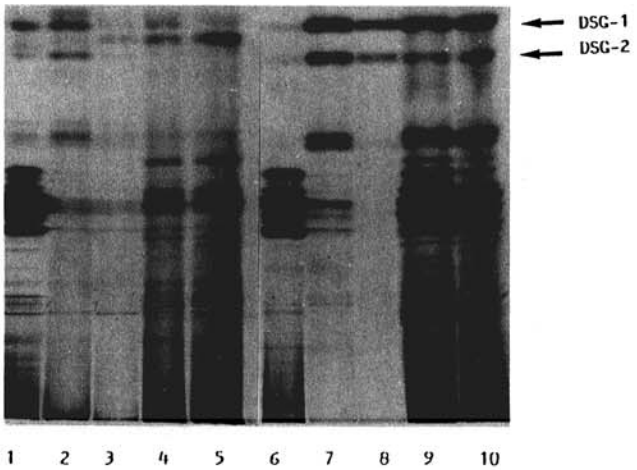

Fig. 6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of bread wheat cultivar Talent (slots 1, 2, 3, 4, and 5) and durum wheat cultivar Mondur (slots 6, 7, 8, 9, and 10) proteins extracted sequentially from gluten with 68% ethanol (1 and 6), 3.75 mg of Na-tetradecanoate (2 and 7) and 80 mg of Na-tetradecanoate (4 and 9) or from flour or semolina with 3.75 mg of Na-tetradecanoate (3 and 8) and 80 mg of Na-tetradecanoate (5 and 10). Extractions were carried out on glutens after the removal of albumins and globulins and on flour and semolina after the sequential removal of albumins-globulins and gliadins with 0.5*M* NaCl and 68% ethanol, respectively. Proteins are not reduced.

of DSG proteins. For bread wheat gluten, these proteins were partly extracted with the gliadins (Fig. 6). The electrophoretic composition of Glu-2 extracted from gluten remained similar to that extracted from flour, although there was an obvious change in proportions among the DSG proteins.

The elution profile of Glu-1 from bread wheat gluten, obtained by chromatographic analysis on Biogel P-30, had many similarities with the elution profile of Glu-1 from durum wheat gluten (Fig. 7), which contained the major proportion of DSG proteins that were present in durum wheat. However, in durum wheat, no differences in the DSG protein region were found between Glu-1 extracted from gluten or from semolina; either their chromatographic profiles or their electrophoretic compositions were similar (Fig. 6). The amino acid composition of peaks 2 plus 3 (Fig. 7) of Glu-1 from bread wheat gluten was quite similar to the amino acid composition of peaks 2, 3, and 4 extracted with 0.5*M* NaCl from bread wheat flour (Table II and Fig. 2). These results show that at least some of the CM-type sulfur-rich proteins that can be extracted from bread wheat flour with the albumin-globulin fraction are readily bound to the other gluten proteins during the preparation of gluten.

Bietz and Wall (1975) found noncovalently bound albumins and globulins in sequentially extracted glutenin. More recently, the presence of low molecular weight, sulfur-rich proteins, called S-proteins, has also been reported in gluten preparations (Zawistowska et al 1985, Zawistowska and Bushuk 1986, Zawistowska et al 1986). In these latter studies, the comparison of chromatographic (on Sephadex G-50) and electrophoretic characteristics of CM proteins with S-proteins showed both similarities and differences. In agreement with these reports, we showed that, in the case of bread wheat, the gluten preparation procedure modified the ease of extraction of low molecular weight, sulfur-rich (CM) proteins. This seemed to occur, at least partly, through hydrophobic interactions between CM-type and gluten proteins, because CM proteins were not washed out with water during the preparation of the gluten, although they could be extracted from gluten with Na-tetradecanoate, which disrupts hydrophobic interactions. In agreement with the findings of Bietz and Wall (1975), covalent bonds were not involved in the modification of protein solubility. However, sulfur-rich, DSG-type proteins may form new S–S bonds during heat treatment (Feillet et al 1989). It has been shown that S-protein can bind considerable amounts of lipid, and has been suggested that the strong associations with

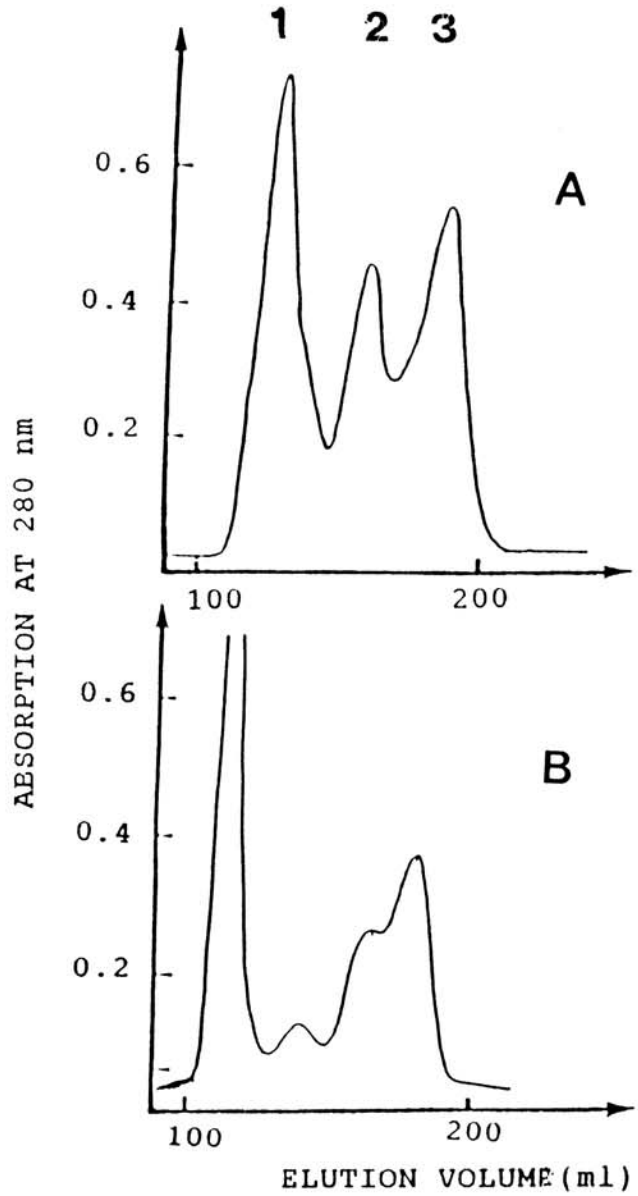

Fig. 7. Elution profiles of proteins extracted with 3.75 mg of Na-tetradecanoate from durum wheat cultivar Mondur (**A**) and bread wheat cultivar Talent (**B**) gluten subsequent to the extraction of gliadins with 68% ethanol.

other gluten proteins occur through lipid-protein and lipid-lipid interactions (Zawistowska et al 1985). Our results can also be explained by such interactions. Strongly bound, specific lipids were found in purified durum wheat DSG proteins (Kobrehel and Sauvaire 1990).

In further preliminary experiments, gluten samples were prepared after the extraction of soluble proteins from flour with water or successively with NaCl and water. The preparation of gluten from the residual protein was very difficult, because the particles did not stick to each other to form a gluten ball. The more exhaustive the extraction of the albumin-globulin fraction (and thus of DSG-type, sulfur-rich proteins), the lower the yield of gluten. The yield of gluten decreased by about 25% after an extraction with water and by about 50% after sequential extractions with NaCl and water. The decrease in gluten yield could partly result from the extraction of the sulfur-rich, CM-type proteins. This would suggest that they have a functional role in gluten adhesion, their presence in the gluten ball being a consequence of their physico-chemical properties. Further experiments are needed to determine whether the various CM-type proteins have a similar functional role and the respective roles of protein and of lipid components.

CONCLUSIONS

Under the extraction conditions used to isolate DSG-1 and DSG-2 (Kobrehel and Alary 1989b), little CM-type, sulfur-rich protein could be extracted from bread wheat. Likewise, the electrophoretic characteristics (on SDS-PAGE and on two-dimensional IEF × SDS-PAGE) of these proteins extracted from bread wheat with the albumin-globulin fraction and the DSG proteins from durum wheat showed considerable differences. Despite their similar N-terminal amino acid sequences, DSG-1 and DSG-2 may not be identical to CM16 and CM3.

Previous studies showed a functional relation between DSG proteins and the cooking quality of durum wheat pasta. Results reported here suggest that CM-type proteins of bread wheat may also have a functional role in the formation of doughs. They seem to interact through hydrophobic interactions with other gluten proteins, glutenins, and gliadins during dough formation and form part of the gluten network. Further investigations are in progress to confirm the functional role of low molecular weight, sulfur-rich proteins in dough formation and to determine the possible specific involvement of each of these proteins in technological processes.

LITERATURE CITED

ALARY, R., and KOBREHEL, K. 1987. The sulfhydryl plus disulfide content in the proteins of durum wheat and its relationship with the cooking quality of pasta. J. Sci. Food Agric. 39:123-136.

ARCHER, J. M. 1979. Interrelationships between rheologically important thiols, disulphide groups and the yield-quality parameters of wheat cultivars (*Triticum aestivum* L.). J. Sci. Food Agric. 30:566-574.

BARBER, D., SANCHEZ-MONGE, R., GARCIA-OLMEDO, F., SALCEDO, G., and MENDEZ, E. 1986. Evolutionary implications of sequential homologies among members of the trypsin/α-amylase inhibitor family (CM proteins) in wheat and barley. Biochim. Biophys. Acta 873:147-151.

BIETZ, J. A., and WALL, J. S. 1975. The effect of various extractants on the subunit composition and association of wheat glutenin. Cereal Chem. 52:145-155.

EWART, J. A. D. 1988. Thiols in flour and breadmaking quality. Food Chem. 28:207-218.

FEILLET, P. 1967. Dosage semi-automatique de la teneur en proteines des cereales par la methode de Kjeldahl. Tech. Ind. Cerealieres. 153:17-19.

FEILLET, P., AIT-MOUH, O., KOBREHEL, K., and AUTRAN, J.-C. 1989. The role of low molecular weight glutenin proteins in the determination of cooking quality of pasta products: An overview. Cereal Chem. 66:26-30.

GARCIA-OLMEDO, F., SALCEDO, G., SANCHEZ-MONGE, R., GOMEZ, L., ROYO, J., and CARBONERO, P. 1987. Plant proteinaceous inhibitors of proteinases and α-amylases. Pages 275-334 in: Oxford Surveys of Plant Molecular and Cell Biology. vol 4. J. B. Miflin, ed. 4:275-334.

GAUTIER, M.-F., ALARY, R., KOBREHEL, K., and JOUDRIER, PH. 1989. Chloroform/methanol-soluble proteins are the main components of *Triticum durum* sulfur-rich glutenin fractions. Cereal Chem. 66:535.

GAUTIER, M.-F., ALARY, R., and JOUDRIER, PH. 1990. Cloning and characterisation of a cDNA encoding the wheat (*Triticum durum* Desf.) CM16 protein. Plant Mol. Biol. 14:313-322.

HUEBNER, F. R., BIETZ, J. A., and WALL, J. S. 1977. Disulfide bonds: Key to wheat protein functionality. Pages 67-88 in: Protein Cross-Linking. Biochemical and Molecular Aspects. M. Friedman, ed. Plenum Press: New York.

KOBREHEL, K., and ALARY, R. 1989a. The role of a low molecular weight glutenin fraction in the cooking quality of pasta. J. Sci. Food Agric. 47:487-500.

KOBREHEL, K., and ALARY, R. 1989b. Isolation and partial characterization of two low molecular weight durum wheat glutenins. J. Sci. Food Agric. 48:441-452.

KOBREHEL, K., and SAUVAIRE, Y. 1990. Particular lipid composition in isolated proteins of durum wheat. J. Agric. Food Chem. 38:1 164-1171.

KOBREHEL, K., REYMOND, C., and ALARY, R. 1988. Low molecular weight durum wheat glutenin fractions rich in sulfhydryl plus disulfide groups. Cereal Chem. 65:65-69.

LEE, C. C., and LAI, T. S. 1967. Studies with radioactive tracers. XI. The use of *N*-ethylmaleimide-1-^{14}C in the determination of flour sulfhydryls and correlations between masked sulfhydryls and loaf volumes. Cereal Chem. 44:620-630.

MAUZE, C., RICHARD, M., and SCOTTI, G. 1972. Guide pratique de controle de la qualite des bles. Institut Technique des Cereales et de Fourrages (ITCF): Paris.

O'FARRELL, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250:4007-4021.

PAYNE, P. I., and CORFIELD, K. G. 1979. Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. Planta 145:83-88.

PAZ-ARES, J., HERNANDEZ-LUCAS, C., SALCEDO, G., ARAGONCILLO, C., PONZ, F., and GARCIA-OLMEDO, F. 1983. The CM proteins from cereal endosperm: Immunochemical relationships. J. Exp. Bot. 34:388-395.

SALCEDO, G., RODRIGUEZ-LOPERENA, A. M., and ARAGONCILLO, C. 1978. Relationships among low MW hydrophobic proteins from wheat endosperm. Phytochemistry 17:1491-1494.

SHEWRY, R. P., LAFIANDRA, D., SALCEDO, G., ARAGONCILLO, C., GARCIA-OLMEDO, F., LEW, J.-L. E., DIETLER, D. M., and KASARDA, D. D. 1984. N-terminal amino acid sequences of chloroform/methanol-soluble proteins and albumins from endosperms of wheat, barley, and related species. FEBS Lett. 175:359-363.

STEVENS, D. J. 1966. The reaction of wheat proteins with sulphite. II. The accessibility of disulphide and thiol groups in flour. J. Sci. Food Agric. 17:202-204.

SULLIVAN, B., DAHLE, L. K., and SCHIPKE, J. H. 1963. The oxidation of wheat flour. IV. Labile and nonlabile sulphydryl groups. Cereal Chem. 40:515-531.

TSEN, C. C., and BUSHUK, W. 1969. Reactive and total sulfhydryl and disulfide contents of flours of different mixing properties. Cereal Chem. 45:58-62.

ZAWISTOWSKA, U., and BUSHUK, W. 1986. Electrophoretic characterization of low-molecular-weight wheat protein of variable solubility. J. Sci. Food Agric. 37:409-417.

ZAWISTOWSKA, U., BEKES, F., and BUSHUK, W. 1985. Gluten proteins with high affinity to flour lipids. Cereal Chem. 62:284-289.

ZAWISTOWSKA, U., BIETZ, A. J., and BUSHUK, W. 1986. Characterization of low-molecular-weight protein with high affinity for flour lipid from two wheat classes. Cereal Chem. 63:414-419.

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